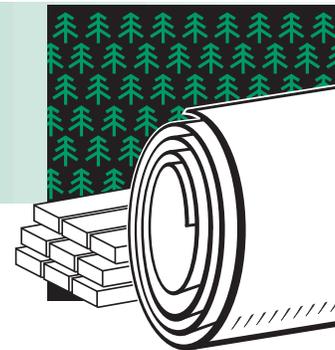


FOREST PRODUCTS

Project Fact Sheet



DEVELOPMENT AND VALIDATION OF STERILITY SYSTEMS FOR TREES

BENEFITS

- Reduces public concerns about genetically engineered forests
- Achieves energy savings in paper production by increasing pulp yield and quality
- Increases carbon sequestration through enhanced vegetative and reduced reproductive growth
- Reduces hazardous by-products if pulp has lowered lignin content
- Lowers the use of pesticides, herbicides, and other toxic compounds, if engineered for these characteristics

APPLICATIONS

Achieving sterility will reduce environmental and legal concerns about the use of transgenic trees for commercial forestry products. The technology is expected to be introduced in 2007, and used on 50 percent of the acreage on which poplar and eucalypts are cultivated for the industry.

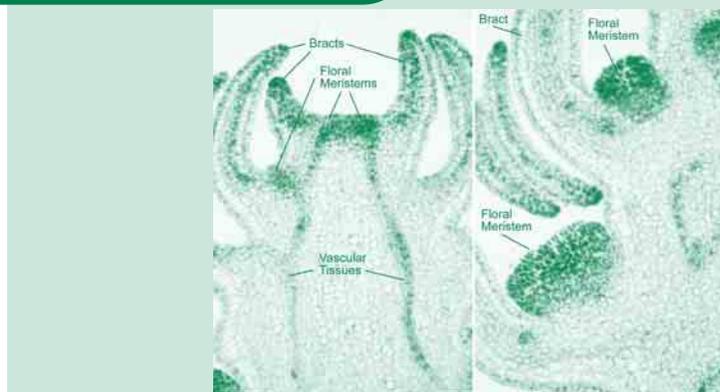
GENETICALLY ENGINEERED STERILITY WILL REDUCE CONCERNS ABOUT TRANSGENIC TREE PLANTATIONS

Genetically modified tree plantations can provide the forestry industry with a source of pulp that provides the desirable characteristics sought in its commercial products (e.g., modified lignin, increased growth rate). Induced sterility may also increase tree growth by avoiding production of reproductive tissue. However, there is widespread concern about the dispersal of the introduced genes into the environment via pollen and seed, and their potential effects on other plant materials. Since U.S. regulations may soon require transgene containment before genetically modified trees may be used commercially, researchers are focusing on inducing genetic sterility in transgenic tree populations.

Because of the difficulties of developing and demonstrating complete sterility in trees such as poplars, the research approach is to study alternate sterility systems in *Arabidopsis* (a small flowering plant often used as a model organism in plant biology) and/or early-flowering tree systems (e.g., eucalypts). Evaluations will be made of several molecular mechanisms designed to engineer sterility, including floral cell ablation and high frequency gene-silencing. Favorable sterility constructs will be tested in transgenic trees in the field.

This four-year project will include matching funds and in-kind support for further field testing of the transgenic trees from the cooperating industrial partnership.

FIGURE 1.



In situ hybridization showing expression of a poplar floral development gene (*PTAP1-1*) in an immature male catkin. *PTAP1-1* expression (dark areas) is seen in floral meristems just as they begin to form and in the developing vascular tissues of the inflorescence stem and bracts. The early and bisexual floral expression of *PTAP1-1* is a strong indication that it acts to specify floral meristem identity in poplar. This gene was isolated under the previous grant, and constructs containing *PTAP1-1* or its promoter will be evaluated in transgenic trees.



Project Description

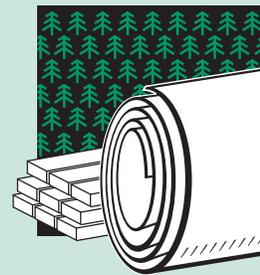
Goal: To develop and validate sterility systems in poplar and eucalyptus trees that meet the requirements for commercial use of transgenic plantations.

Floral regulatory genes and transgenic poplars have been under study for more than 10 years in the principal investigators' laboratory. The current effort is a continuation of a previously awarded project, "Dominant Negative Mutations (DNMs) of Floral Homeotic Genes for Genetic Engineering of Sterility in Forest Trees."

In this project, approaches to genetic engineering of sterility will include (1) dominant negative mutant transgenes (genes that encode mutant proteins to block the activity of wild-type proteins); DNM constructs will be evaluated in *Arabidopsis*, poplar, and early-flowering eucalypts; (2) gene-silencing transgenes (double-stranded RNA that induces silencing of endogenous genes after transcription, in some cases by 100 percent); and (3) floral ablation transgenes (a floral promoter that directs the expression of a cytotoxin in floral cells).

Progress & Milestones

- Researchers have isolated and characterized six poplar gene homologs of well-studied *Arabidopsis* genes that control early flower development.
- Introduction of a poplar promoter for floral ablation resulted in perturbation or complete ablation of flowers in transgenic *Arabidopsis*, tobacco, and early-flowering poplar.
- Following analysis of the second generation of *Arabidopsis* DNMs during the first year of this project, a decision will be made about whether to proceed with production and testing of poplar DNM constructs.
- Efforts will focus on isolating a poplar gene homolog from *Arabidopsis* necessary for both male and female plant fertility, which acts during late flower development and may allow floral structures to be maintained while imparting sterility.
- Researchers will seek to verify that the sterility is complete and stable over multiple growing seasons, that it does not interfere with the plants' vegetative growth, and hope to develop a method that enables the transformation to sterility to be identifiable at the molecular level in juvenile trees.
- Redundant sterility systems will be established by combining the best genes for sterility that act by distinct mechanisms, or that target different genes.
- If laboratory and greenhouse studies identify a potentially effective sterility system, field tests will be conducted on transgenic poplars.



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